Claims

1. A method for the joint and in each case specific detection of a mycobacterial infection, of the *Mycobacterium tuberculosis* complex and/or of *Mycobacterium avium* in clinical material comprising the steps of

a) extraction of microbial DNA from clinical material,

b) amplification of at least one fragment of the 16S rRNA gene from the extracted DNA by means of a primer pair including the nucleotide sequences SEQ ID NO: 1/SEQ ID NO: 5 or by means of two primer pairs, where one primer pair includes the nucleotide sequence SEQ ID NO: 2/SEQ ID NO: 3 and the other primer pair includes the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 5,

c) detection of the genus-specific region of the amplified 16S rRNA fragment of mycobacteria by means of a pair of labelled hybridization probes, where the pair includes the nucleotide sequences SEQ ID NO: 10/SEQ ID NO: 11,

d) detection of the species-specific region of the amplified 16S rRNA fragment of mycobacteria by means of a pair of labelled hybridization probes, where the pair includes the nucleotide

sequences SEQ ID NO: 6/SEQ ID NO: 7 or the complementary sequences thereof for detecting the *Mycobacterium tuberculosis* complex, and where the pair includes the nucleotide sequences of SEQ ID NO: 8/SEQ ID NO: 9 or the complementary sequences thereof for detecting *Mycobacterium avium*, and

e) where the joint, in each case specific detection of mycobacteria and of the *Mycobacterium tuberculosis* complex and/or of *Mycobacterium avium* takes place during the detection as in steps c) and d) by means of melting curve analysis.

2. The method according to claim 1, where the extracted microbial DNA is mixed in step a) of the method with at least one artificial plasmid which serves as internal standard, where the artificial plasmid includes a genus-specific region III of the 16S rRNA of mycobacteria or parts thereof, which has been modified in its nucleotide sequence, and where a melting curve analysis is carried out for specific detection of the amplified 16S rRNA fragments of mycobacteria and of the modified 16S rRNA fragments of the artificial plasmid.

3. The method according to claim 1, where the extracted microbial DNA is divided and a first portion thereof is further treated in the method specified in steps a) to e) of Claim 1, and a second portion is subjected to a parallel method comprising the steps of

a')mixing of the extracted microbial DNA with at least one artificial plasmid as internal standard, where the artificial plasmid includes a genus-specific region III of the 16S rRNA of mycobacteria or parts thereof, which has been modified in its nucleotide sequence,

b')amplification of the 16S rRNA fragment by means of at least one primer pair selected from the group including the nucleotide sequence pairs SEQ ID NO: 1/SEQ ID NO: 5 and SEQ ID NO: 4/SEQ ID NO: 5,

c')detection of the amplified 16S rRNA fragments by means of a pair of labelled hybridization probes which hybridize with the genus-specific region III of the 16S rRNA fragment of mycobacteria, where the pair includes the nucleotide sequences SEQ ID NO: 10/SEQ ID NO: 11 or the complementary sequences thereof, and

d')where a melting curve analysis takes place for the specific detection of the amplified 16S rRNA fragments of mycobacteria and of the modified 16S rRNA fragments of the at least one artificial plasmid during the detection as in step c').

4. The method according to any of the preceding claims, where the amplification of the gene fragments is carried out by means of the polymerase chain reaction (PCR).

5. The method according to claim 4, where the polymerase chain reaction (PCR) is carried out as

real-time PCR, preferably by means of the LightCycler™ system.

6. The method according to any of the preceding claims, where the detection takes place during or after the amplification of the 16S rRNA fragments.

7. The method according to any of the preceding claims, where the detection takes place by means of real-time PCR, particularly preferably by means of the LightCycler™ system.

8. The method according to any of the preceding claims, where the detection is carried out by means of fluorescence detection, and where the labelled hybridization probe pairs are configured as fluorescence resonance energy transfer pair.

9. The method according to any of the preceding claims, where the melting curve analysis takes place following the amplification of the 16S rRNA fragments by means of real-time PCR, preferably by means of the LightCycler™ system.

10. The method according to claim 8 or 9, where the detection is carried out as quantitative measurement.

11. The method according to any of the preceding claims, where the clinical material is selected from the group of clinical samples consisting of sputum, bronchial lavage, gastric juice, urine, stool, liquor, bond marrow, blood and biopsies.

12. An oligonucleotide primer pair which comprises the nucleic acids with the nucleotide sequences SEQ ID NO: 2 and SEQ ID NO: 3.

13. An oligonucleotide primer which comprises the nucleic acids with the nucleotide sequence SEQ ID NO: 4.

14. An oligonucleotide hybridization probe pair which comprises the nucleic acids with the nucleotide sequences SEQ ID NO: 6 and SEQ ID NO: 7 or with the complementary sequences thereof.

15. An oligonucleotide hybridization probe pair which comprises the nucleic acids with the nucleotide sequences SEQ ID NO: 8 and SEQ ID NO: 9 or with the complementary sequences thereof.

16. An oligonucleotide hybridization probe pair which comprises the nucleic acids with the nucleotide sequences SEQ ID NO: 10 and SEQ ID NO: 11 or with the complementary sequences thereof.

17. An artificial plasmid which can be employed as internal control of the amplification and of the detection of 16S rRNA fragments of mycobacteria, including modified sequences of the genus-specific region III of the 16S rRNA gene.

18. An artificial plasmid according to claim 17, where the artificial plasmid comprises at least one nucleotide exchange, addition, deletion and/or inversion vis-à-vis the wild-type se-

quence of the genus-specific region III of the 16S rRNA gene.

19. An artificial plasmid as claimed in Claim 17 or 18, including the nucleotide sequence SEQ ID NO: 14 or SEQ ID NO: 15.

20. An artificial plasmid as claimed in Claim 17 or 18, including the nucleotide sequence SEQ ID NO: 16 or SEQ ID NO: 17.

21. A diagnostic kit for the specific detection of a mycobacterial infection and of the *Mycobacterium tuberculosis* complex and of *Mycobacterium avium* in clinical material by the method according to any of claims 1 to 11 including:

a) at least one polymerase,

b) at least one primer pair according to claim 12,

c) at least one primer pair comprising a primer according to claim 13 which amplifies the genus-specific region III of mycobacteria.

d) a hybridization probe pair according to claim 16 for the detection of the genus-specific region III,

e) at least one hybridization probe pair according to claims 14 to 15 for the detection of the species-specific regions.

22. A diagnostic kit according to claim 21, comprising an artificial plasmid according to any of claims 17 to 20.